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Short communication

Label-free detection of low protein concentration in solution using a novel colorimetric assay

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ABSTRACT

Dual pH and temperature sensitive microgel-based etalons were fabricated by sandwiching a "monolithic" microgel layer between two semitransparent, Au layers. The devices exhibit visual color and multipeak reflectance spectra, both of which primarily depend on the distance between the Au surfaces mediated by the microgel diameter. We found that a polycationic polyelectrolyte can penetrate through the Au overlayer to interact with negatively charged microgel confined between Au overlayers. In this submission we report that biotinylated polycationic polymer can penetrate through the Au overlayer of a poly (*N*-isopropylacrylamide)-co-acrylic acid (pNIPAm-co-AAc) microgel-based etalon and cause the microgel layer to collapse. The collapse results in a shift in the spectral peaks of the reflectance spectra. We found that the extent of peak shift depends on the amount of biotinylated polycation added to the etalon, which can subsequently be used to determine the concentration of streptavidin in solution at pM concentrations.

1. Introduction

To properly diagnose and treat disease, low concentrations of DNA and/or protein in e.g., blood, urine, and cerebral spinal fluid, need to be detected. Advances over recent years have allowed increasingly low levels of the biological markers of disease (biomarkers) to be detected (Elghanian et al., 1997; Haes et al., 2005; Hall et al., 2011; Nam et al., 2003; Shiddiky et al., 2012; Wang et al., 2009; Zhu et al., 2010). This has had an enormous impact on the quality of life of individuals as it has allowed for early diagnosis and treatment of disease before symptoms emerge.

In developed countries, receiving a diagnosis and subsequent treatment is relatively facile compared to developing countries. For example, the inability of a patient to reach a hospital for a diagnosis can result in death. As a result, diagnostic devices that are capable of functioning in environments without running water, electricity, and temperature control are being developed. These devices, typically referred to as point-of-care (POC) diagnostics, also need to be reliable, inexpensive, easy to operate/results easily interpreted, and fast (Bonanno and DeLouise, 2010; Yager et al., 2008). In a continuing effort to develop new, more sensitive and selective POC diagnostic devices new materials are being developed.

Currently, polymer-based materials are primarily used for biological applications (Anderson et al., 2004; Holtz and Asher, 1997; Kim et al., 2005, 2006; Langer,1998; Plunkett et al., 2005; Sharma et al., 2004; Tauro and Gemeinhart, 2005). This is partially due to the tremendous chemical diversity of polymer backbones. They are also easily modified with biomolecules, e.g., proteins and DNA using mild reaction conditions (Dong et al., 2007). Of specific interest to this manuscript are responsive polymer-based materials. For example, responsive polymers are able to respond to changes in their environment by undergoing conformational changes (Gutowska et al., 1994; Kwon et al., 1991; Palankar and Skirtach, 2009; Pelton, 2000; Suzuki and Tanaka, 1990). The most common stimuli include temperature, pH, concentration, ionic strength, light, electric and magnetic field, and redox (Ayano et al., 2012; Palankar and Skirtach, 2009). Usually these responsive polymers exhibit reversibility. That is, once the stimulus is removed from the system, the polymers return back to their initial state (Gutowska et al., 1994; Kwon et al., 1991; Pelton, 2000; Suzuki and Tanaka, 1990). Ideally, the reversible response can be achieved over a large number of cycles without compromising their response to stimuli.

The most extensively studied responsive polymer is poly (*N*-isopropylacrylamide) (pNIPAm). PNIPAm is fully water soluble at $T < 32$ °C, and exists in water as a fully solvated random coil. At $T > 32$ °C, pNIPAm "dehydrates" due to favorable chain–chain interactions, and transitions to a dense globule (Wang et al., 1998). This temperature is referred to as the lower critical solution temperature (LCST). Furthermore, the transition is fully reversible. The great advantage of pNIPAm-based polymers is that their transition temperature is close to body temperature, and it can be easily copolymerized with other monomers to acquire additional

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functionality to stimuli other than temperature (Brazel and Peppas, 1995).

The synthesis of crosslinked pNIPAm-based networks (hydrogels) as well as pNIPAm-based hydrogel particles (nano or microgels, depending on their dimensions) is also well known. Like linear pNIPAm, pNIPAm-based hydrogels and microgels decrease in size, and dehydrate, at $T > LCST$. Likewise, this transition is reversible. Finally, they too can also be chemically modified such that they are responsive to multiple stimuli (Hendrickson et al., 2010). The most common additional responsivity added to pNIPAm-based microgels is pH. This is typically accomplished by copolymerization of acrylic acid (AAc) into the microgel during synthesis (Hoare and Pelton, 2004; Kim et al., 2004). The pKa value of acrylic acid is ~4.25, i.e., increasing the pH of a solution above 4.25 induces the deprotonation of AAc and thus makes the microgel particles polyanionic in nature. This, in turn, makes the microgel's diameter sensitive to the presence of polycations (Kleinen and Richtering, 2011). For example, Richtering and coworkers (Kleinen and Richtering, 2011) reported the size change of polyanionic poly (*N*-isopropylacrylamide-co-methacrylic acid) pNIPAm-co-MAAc microgels in the presence of polycationic, polydiallyldimethylammonium chloride (pDADMAC). They hypothesized the size change was a result of pDADMAC penetration into the microgel structure, resulting in intramolecular crosslinking of the microgel's charged polymer chains.

We have shown that pNIPAm microgel-based optical devices (or etalons) can be fabricated by sandwiching pNIPAm-based microgels between two thin Au layers. This is shown schematically in Fig. 1a. These devices show visible color and multipeak reflectance spectra, as seen in Fig. 1b. The position of the peaks in the reflectance spectra is dependent on the distance between two Au layers and the refractive index of microgel (Sorrell et al., 2011a, 2011b). The position and order of the peaks can be predicted from Eq. (1):

$$\lambda = 2nd \cos\theta / m \quad (1)$$

where n is the refractive index of the microgel (dielectric) layer, d is the mirror–mirror distance, θ is the angle of incident light relative to the normal, and m (an integer), is the order of the reflected peak. We have shown that these devices change color and peak position with pH, temperature and in the presence of glucose. In each case, the change in the reflectance spectrum is a direct result of the change in the solvation state (and "diameter") of the microgel in the presence of the stimuli. As a result, the distance between the Au mirrors changes leading to a change in λ of the reflectance peak(s) (Sorrell and Serpe, 2011, 2012). For example, for sensing glucose in solution, pNIPAm-co-AAc microgels were functionalized with 3-aminophenylboronic acid, which binds glucose. Under the assay conditions, when glucose binds to the microgels the charge density in the microgel-based cavity increases, leading to an increase in the cavity thickness and a concomitant red shift in reflectance spectrum. In this submission, we show that polyelectrolyte-induced crosslinking (and subsequent collapse) of the etalon's microgel layer can be used for detecting the concentration of streptavidin in aqueous solutions.

2. Materials and methods

2.1. Materials

N-Isopropylacrylamide was purchased from TCI (Portland, Oregon) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ) prior to use. N,N′-methylenebisacrylamide (BIS) (99%), acrylic acid (AAc) (99%), ammonium persulfate (APS) (98.5%), and poly(allylamine hydrochloride) (PAH, MW = 58,000) were obtained from Sigma-Aldrich (Oakville, ON) and were used as received. NHS-Biotin ((+)-Biotin N-hydroxysuccinimide ester) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium chloride and sodium hydroxide were obtained from Fisher (Ottawa, ON). All deionized (DI) water was filtered to have a resistivity of 18.2 MΩ cm and was obtained from a Milli-Q Plus system from Millipore (Billerica, MA). Chromium (Cr) and Gold (Au) were deposited using a model THEUPG thermal evaporation system from Torr International Inc. (New Windsor, NY). The annealing of Cr/Au layer was done in a Thermolyne muffle furnace from Thermo Fisher Scientific (Ottawa, Ontario). Anhydrous ethanol was obtained from Commercial Alcohols (Brampton, Ontario). Sodium Hydroxide (NaOH, 99.8%) and hydrochloric acid were purchased from Caledon Chemicals (Georgetown, Ontario) and were used as received. Fisher's finest prewashed glass coverslips were 25 × 25 mm and obtained from Fisher Scientific (Ottawa, Ontario). Cr (99.999%) was obtained from ESPI (Ashland, OR), while Au (99.99%) was obtained from MRCS Canada (Edmonton, AB). EDC (1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride), Biotin-modified magnetic particles (average diameter of < 50 nm) were purchased from TurboBeads (Magdalenenstrasse, Zurich).

2.2. Procedures

2.2.1. Poly (*N*-isopropylacrylamide-co-acrylic acid) (pNIPAm-co-AAc) microgel synthesis

Microgels composed of poly (*N*-isopropylacrylamide-co-acrylic acid) (pNIPAm-co-AAc) were synthesized via temperature-ramp, surfactant free, free radical precipitation polymerization as described previously (Sorrell et al., 2011a, 2011b; Sorrell and Serpe, 2011).

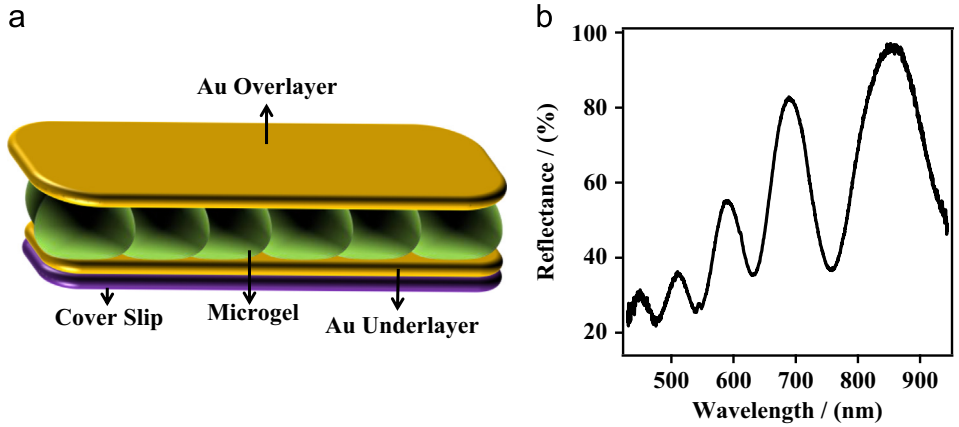

Fig. 1. (a) Basic structure of a microgel-based etalon. (b) Representative reflectance spectrum of a pNIPAm microgel-based etalon soaked in water.